Intrac QP Kit
for expression and detection of protein–protein interactions *in vivo* in mammalian cells

Instruction Manual

Catalogue # K1-220-001

CONTENTS

Components of the Intrac QP Kit

Catalogue #	*Product Name*	*Component*	*Description*	*Concentration (µg/µl)*	*Amount (µl)*
K1-220-001	**Intrac QP kit***				
P1-106-020 P1-107-020 P1-108-020		pQM-CMV-E2Tag-N-A (B, C)	Expression vectors for N-terminus E2-tagging in 3 reading frames	0,5	20
P1-109-020 P1-110-020 P1-111-020		pQM- CMV-E2Tag-C-A (B, C)	Expression vectors for C-terminus E2-tagging in 3 reading frames	0,5	20
P1-112-020		pQM- CMV-E4Tag-N-A	Expression vectors for N-terminus E4-tagging in 3 reading frames	0,5	20
P1-113-100		pQM- CMV-E4Tag-C-A	Expression vectors for C-terminus E4-tagging in 3 reading frames	0,5	20
R1-100-500		3F12 matrix**	Sepharose 4B Fast Flow conjugated with 3F12 antibody, 50% suspension		500
A1-150-100		1E2	Anti-E4Tag mouse monoclonal antibody	1,0	100
A1-100-100		5E11	Anti-E2Tag mouse monoclonal antibody	1,0	100
A1-900-050		HRP	Anti-mouse lgG HRP-conjugate	1,0	50
A1-902-050		AP	Anti-mouse lgG AP-conjugate	1,0	50

* Every component of the Intrac QP Kit is available separately.

Shipping and Storage

The Intrac QP Kit is shipped on ice.
** 3F12 matrix store at +4°C, other components store at –20°C.
All the kit components are stable until the expiry date indicated (see lot-specific label imprint).

Product Use Limitation

The Intrac QP Kit is developed, designed and sold for research purpose and *in vitro* use only.
The product is not to be used for diagnostic procedures.

Additional Materials Required

BCIP/NBT Solution for AP
ECL detection kit
PVDF or Nitorcellulose (NCF) Membranes

Notification to Purchaser

The CMV promoter is covered under U. S. Patents 5,168,062 and 5,385,839 and its use is permitted for research purpose only. Any other use of CMV promoter requires a licence from the University of Iowa Research Foundation, 214 Technology Innovation Center, Iowa City, IA 52242.

Ordering Information and Technical Service

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Introduction

Epitope tagging is a technology that can be effectively used to express, detect, characterize and purify recombinant proteins.
The E2Tag is a new epitope, the peptide that is derived from Bovine Papillomavirus type-1 transactivator protein E2 and consists of 10 amino acid (SSTSSDFRDR).
The E4Tag is also derived from Bovine Papillomavirus type-1 transactivator protein E2 and consists of 10 amino acid (GTTGHYSVRD).
The Intrac QP kit is designed for detection protein:protein interaction in vivo in mammalian cells. The coding sequences of the tested proteins are cloned into the two expression vectors so that these are signed differently by the E2-tag or by the E4-tag, respectively. Followed co-transfection of the plasmids allows expression of the tagged proteins in the cells. If in vivo interaction of the proteins is occurred, it could be detected by co-immunoprecipitation by anti-E2tag antibody matrix.

The Intrac QP Kit consists of four pQM vectors and a 3F12-matrix suspension, a monoclonal anti-E2Tag antibody, a monoclonal anti-E4Tag antibody and secondary antibodies conjugated with HRP and AP.

The pQM-E2Tag vectors provide E2Tag epitope tagging options, which allow E2Tag epitope tagging at either the N- or C-terminus of the protein. The pQM-E4Tag vectors provide E4Tag epitope tagging options, which allow E4Tag epitope tagging at either the N- or C-terminus of the protein. The 3F12-matrix suspension allows catch the protein complex through the E2 epitope.

A mouse monoclonal anti-E2Tag antibody recognizes recombinant E2-tagged proteins. A mouse monoclonal anti-E4Tag antibody recognizes recombinant E4-tagged proteins. For detection of E2-tagged or E4-tagged recombinant proteins by immunoblot analysis anti-E2Tag antibody will be visualized with HRP, or AP-conjugated secondary goat anti-mouse (lgG) antibodies.

Expression Vectors

Mammalian expression is driven by the CMV immediate early promoter. The polyadenylation sequence provides signals required for termination of mammalian transcription and translation.
pQM-E2Tag-N and pQM-E4Tag-N vectors are for the cloning of E2Tag and E4Tag at the N-terminus of the proteins.
pQM-E2Tag-C and pQM-E4Tag-C vectors are for the cloning of E2Tag and E4Tag at the C-terminus of the proteins.
The translational start sequence used in pQM-E2Tag-N is a Kozak consensus sequence GCC**ATG**G and in pQM-E4Tag-N GCCACC**ATG.** pQM-E2Tag-C and pQM-E4Tag-C vectors do not contain any translation start sequence.

We recommend cloning the gene of interest into the pQM vectors using restriction enzymes indicated in MCS.

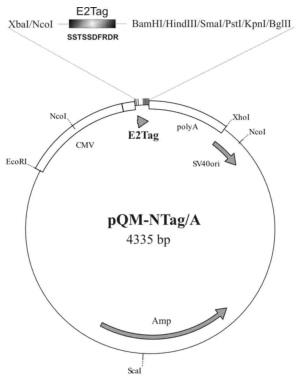
Vector Information

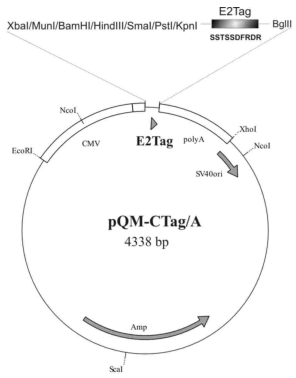
pQM-E2Tag-N and pQM-E4Tag-N 4335 bps

Nucleotide position	Description
1-444	polyA
444-644	SV40ori
2503-1643	Amp
3584-4189	CMV
4190-4259	tk leader
4275-4304	E2Tag or E4Tag
4305-5	Multiple Cloning Site (MCS)

pQM-E2Tag-C-A and pQM-E4Tag-C 4338 bps

Nucleotide position	Description
42-71	E2Tag
79-522	polyA
522-722	SV40ori
2581-1721	Amp
3662-4267	CMV
4268-4337	tk leader
6-41	Multiple Cloning Site (MCS)





E2Tag

XbaI NcoI ScaI
TCT AGA GCC **ATG** GGT **TCA AGT ACT TCT TCT GAT TTT AGA GAT CGC**
Met Gly **Ser Ser Thr Ser Ser Asp Phe Arg Asp Arg**

BamHI HindIII SmaI PstI KpnI BglII

pQM-NTag/A GGA TCC AAG CTT CCC GGG CTG CAG GGT ACC AGA TCT
Gly Ser Lys Leu Pro Gly Leu Gln Gly Thr Arg Ser

BamHI HindIII SmaI PstI KpnI BglII

pQM-NTag/B GGG ATC CAA GCT TCC CGG GCT GCA GGG TAC CAG ATC T
Gly Ile Gln Ala Ser Arg Ala Ala Gly Tyr Gln Ile

BamHI HindIII SmaI PstI KpnI BglII

pQM-NTag/C GCG GAT CCA AGC TTC CCG GGC TGC AGG GTA CCA GAT CT
Ala Asp Pro Ser Phe Pro Gly Cys Arg Val Pro Asp

XbaI MunI BamHI HindIII SmaI PstI KpnI

pQM-CTag/A TCT AGA CAA TTG GGA TCC AAG CTT CCC GGG CTG CAG GGT ACC
Ser Arg Gln Leu Gly Ser Lys Leu Pro Gly Leu Gln Gly Thr

XbaI MunI BamHI HindIII SmaI PstI KpnI

pQM-CTag/B T CTA GAC AAT TGG GAT CCA AGC TTC CCG GGC TGC AGG GTA CCC
Leu Asp Asn Trp Asp Pro Ser Phe Pro Gly Cys Arg Val Pro

XbaI MunI BamHI HindIII SmaI PstI KpnI

pQM-CTag/C TC TAG ACA ATT GGG ATC CAA GCT TCC CGG GCT GCA GGG TAC CTA
STOP Thr Ile Gly Ile His Ala Ser Arg Ala Ala Gly Tyr Leu

E2Tag

ScaI BglII
TCA AGT ACT TCT TCT GAT TTT AGA GAT CGC AAC **TGA** AGA TCT
Ser Ser Thr Ser Ser Asp Phe Arg Asp Arg Asn **STOP**

For complete vector sequence please visit our website at www.quattromed.com

Monoclonal Antibodies

Anti-E2Tag monoclonal antibody
Clone: 5E11
Subtype: Mouse IgG1
Concentration: 1.00 mg/ml
Specifity: recognizes proteins containing the sequence SSTSSDFRDR
Preparation: purified from mouse ascites fluid, dialyzed against phosphate buffered saline (PBS) and adjusted to the concentration 1 mg/ml.
Storage buffer: PBS + 50% glycerol.
Working concentration: Recommended dilution for Western blot analysis 1: 5 000 - 1:1000 (a concentration of 0.2 – 1.0 µg antibody/ml).

Anti-E4Tag monoclonal antibody
Clone: 1E2
Subtype: Mouse IgG1
Concentration: 1.00 mg/ml
Specifity: recognizes proteins containing the sequence GTTGHYSVRD
Preparation: purified from mouse ascites fluid, dialyzed against phosphate buffered saline (PBS) and adjusted to the concentration 1 mg/ml.
Storage buffer: PBS + 50% glycerol.
Working concentration: Recommended dilution for western blot analysis 1:5.000 – 1: 1000 (a concentration of 0.2 – 1.0 µg antibody/ml).

Secondary Antibodies

Anti-mouse IgG HRP-conjugate, goat anti-mouse IgG affinity purified antibody, peroxidase conjugated
Storage buffer: 0.15 M PBS pH 7.4, containing 0.1% sodium azide and 50% glycerol
Working concentration: Recommended dilution for western blot analysis is 1:10.000

Anti-mouse IgG AP-conjugate, goat anti-mouse IgG affinity purified antibody, alkaline phosphatase conjugated
Storage buffer: 0.15 M PBS pH 7.4, containing 1 mM $MgCl_2$, 0.1% sodium azide and 50% glycerol
Working concentration: Recommended dilution for western blot analysis is 1:5000

Protocols

Preparing cell lysate

1. Appropriate amounts of the expression vector DNAs are co-transfected to cultured mammalian cells using suitable protocol. High expression levels are obtained in SV40 virus transformed Cos-7 cells, probably caused by SV40 origin placed into the vector. Principally, most mammalian cell lines could be usable because strong and universal CMV IE promoter is introduced into the vectors for driving the expression.
2. The cells are usually harvested 2-4 days post-transfection. After washing twice with PBS, the cells are collected and lysed in native lysis buffer. The composition of the buffer as well lysis conditions may need optimization for each experiment. In our assays, the cells were lysed at 30 minutes on ice in the buffer:
   50mM TrisHCl (pH 7.5)
   2mM EDTA (pH 8.0)
   0,5% IGEPAL
   10mM NaCl
   1mM PMSF
   1µg/µl aprotinin
   1µg/µl leupeptin

Next to the lysis, insoluble material are precipitated by centrifugation 10.000xg at 10 minutes and cleared lysate are collected into the new tube.

3F12-matrix

1. Equilibrate the matrix 2x5 volumes with buffer A:
   i. 50mM TrisHCl pH 7,5
   ii. 2mM EDTA
   iii. 10mM NaCl
   iv. 1mM PMSF
2. Suspend the matrix, spin at 190x g, remove the supernatant

Binding to the 3F12-matrix

Equilibrated 3F12-matrix is incubated with cell lysate at 4°C 1H until overnight.

1. Mixture is loaded to the column.
2. To the removal non-bound material the column is centrifuged at 3000Xg for 20 seconds.
3. Column is subsequently washed with buffers A (composition is described in previous section) and buffer B:
   i. 100mM NaCl
   ii. 2mM EDTA
   iii. 50mM Tris-HCl pH 7,5
   iv. 0,5% glycerol
   v. 1mM PMSF
   vi. 1µg/ml aprotonin
   vii. 1µg/ml leupeptin

Elution of the proteins from the matrix

For elution of immunoprecipitated proteins from the matrix, treat it with SDS gel loading buffer (Laemmli buffer) followed and short centrifugation for removing matrix.

Western blot

1. After electrophoresis, blot the gel onto a NCF or PVDF membrane using a standard Western blot protocol.
2. Block the membrane for 1 hour at room temperature with 2% non-fat dry milk in Tris-buffered saline, 0.05% Tween 20, pH 7.5 (TBST).
3. Wash the membrane once with TBST.
4. Dilute anti-E2Tag antibody to a concentration of 0.2-1.0 µg/ml in diluent containing 2% non-fat dry milk in TBST. If you have problems with high background, NaCl can be added up to a concentration of 1.5 M. Incubate membrane with diluted anti-E2Tag antibody for 1 hour at room temperature with gentle rotation.
5. Wash the membrane three times, 15 min per wash, with TBST.
6. Dilute goat anti-mouse-HRP conjugate diluted 1:10,000 in diluent (2% non-fat dry milk in TBST) or goat anti-mouse-AP conjugate diluted 1:5000 in diluent (2% non-fat dry milk in TBST), and incubate the membrane for 1 hour at room temperature with gentle rotation.
7. Wash the membrane four times, 15 min per wash, with TBST.
8. Detect the signal using an ECL detection kit or NBT/BCIP reagents according to the manufacturer's manual and standard protocols.

References

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4. Koljak, R., et al., The basis of prostaglandin synthesis in coral, J. Biol. Chem., 276: 7033-7040, 2001
5. Örd, D., Örd, T., Mouse NIPK interacts with ATF4 and affects its transcriptional activity, Exp. Cell Res., 286: 308-320, 2003